

CLAIMS

1. A method for detecting and quantitating a micro-
organism having a specific function and its gene from the
5 natural environment, the method comprising the steps of:

1) subjecting a microorganism-containing sample
collected from the natural environment to serial dilution, then
incubating it under the conditions under which the
microorganism having a specific function can grow, and then
10 counting the grown microorganism having a specific function,
and in parallel with these operations, counting the total
number of microorganisms in the above microorganism-
containing samples, and simultaneously counting the total
number of heterotrophic microorganisms, and estimating the
15 dominant level of the microorganism having a specific function
in the natural environment from the ratio of the number of the
microorganism having a specific function to the total number
of microorganisms and/or of heterotrophic microorganisms;

2) extracting DNA from the microorganism in a liquid
20 culture broth of the highest dilution ratio at which the growth
of the microorganism is judged as positive, and amplifying
specific gene domains using said DNA as templates, followed
by cloning;

3) examining the difference of the gene domains thus
25 cloned, and determining the nucleotide sequences thereof; and

4) identifying the microorganism having a specific function inhabiting the natural environment from the nucleotide sequences data thus determined.

2. A method as claimed in Claim 1, wherein the number
5 of the microorganism having a specific function and the total number of heterotrophic microorganisms is counted by an MPN method, the total number of the microorganisms is counted by a direct microscopic counting method, and the growth of the microorganism having a specific function is judged by
10 observation under a microscope.

3. A method as claimed in Claim 1, wherein the microorganism having a specific function is a microorganism which degrades a specific chemical substance.

4. A method as claimed in Claim 2, wherein the
15 microorganism having a specific function is a microorganism which degrades a specific chemical substance.

5. A method as claimed in Claim 3, wherein the specific chemical substance is a harmful chemical substance.

6. A method as claimed in Claim 4, wherein the specific
20 chemical substance is a harmful chemical substance.

7. A method as claimed in Claim 3, wherein the specific chemical substance includes petroleum and petroleum components.

8. A method as claimed in Claim 4, wherein the specific
25 chemical substance includes petroleum and petroleum

components.

9. A method for assessing the function of a microbial population in the natural environment by analyzing succession of the microorganism existing predominantly in the natural
5 environment using the method as claimed in Claim 1.

10. A method for assessing the function of a microbial population in the natural environment by analyzing succession of the microorganism existing predominantly in the natural environment using the method as claimed in Claim 2.

11. A method for analyzing and assessing a polluted environment using the method as claimed in Claim 1.

12. A method for analyzing and assessing a polluted environment using the method as claimed in Claim 2.

13. A method for analyzing and evaluating an environment
15 polluted by harmful chemicals using the method as claimed in Claim 3.

14. A method for analyzing and evaluating an environment polluted by harmful chemicals using the method as claimed in Claim 4.

20 15. A method for analyzing and evaluating an environment polluted by harmful chemicals using the method as claimed in Claim 5.

16. A method for analyzing and evaluating an environment polluted by harmful chemicals using the method as claimed in
25 Claim 6.

17. A method for analyzing and evaluating an oil-polluted environment using the method as claimed in Claim 7.

18. A method for analyzing and evaluating an oil-polluted environment using the method as claimed in Claim 8.

5 19. A method as claimed in Claim 1, wherein the microorganism having a specific function is a microorganism producing a useful enzyme.

20. A method as claimed in Claim 2, wherein the microorganism having a specific function is a microorganism
10 producing a useful enzyme.

21. A 16S rDNA having the nucleotide sequence represented by any of SEQ ID NOS: 1 to 4.

22. An RNA or DNA probe with the length of from 10 to 50 bases which has a part of the nucleotide sequence represented
15 by any of SEQ ID NOS: 1 to 4 and is hybridizable specifically with a petroleum-degrading bacterium belonging to the genus *Cycloclasticus*.

23. An RNA or DNA probe as claimed in Claim 22, wherein the part of the nucleotide sequence represented by any of SEQ
20 ID NOS: 1 to 4 is selected from the group consisting of the nucleotide sequences represented by SEQ ID NOS: 5, 6 and 7.

24. An RNA or DNA probe as claimed in Claim 22, which is used in detection or quantification of a petroleum-degrading bacterium belonging to the genus *Cycloclasticus*.

25 25. An RNA or DNA probe as claimed in Claim 23, which

is used in detection or quantification of a petroleum-degrading bacterium belonging to the genus *Cycloclasticus*.

26. An RNA or DNA probe as claimed in Claim 24, wherein the petroleum-degrading bacterium belonging to the genus
5 *Cycloclasticus* is *Cycloclasticus pugetii* or its closely related species.

27. An RNA or DNA probe as claimed in Claim 25, wherein the petroleum-degrading bacterium belonging to the genus
10 *Cycloclasticus* is *Cycloclasticus pugetii* or its closely related species.

28. An RNA or DNA probe as claimed in Claim 22, which is used in screening a petroleum-degrading bacterium belonging to the genus *Cycloclasticus*.

29. An RNA or DNA probe as claimed in Claim 23, which
15 is used in screening a petroleum-degrading bacterium belonging to the genus *Cycloclasticus*.

30. An RNA or DNA probe as claimed in Claim 28, wherein the petroleum-degrading bacterium belonging to the genus
20 *Cycloclasticus* is *Cycloclasticus pugetii* or its closely related species.

31. An RNA or DNA probe as claimed in Claim 29, wherein the petroleum-degrading bacterium belonging to the genus
Cycloclasticus is *Cycloclasticus pugetii* or its closely related species.

25 32. A method for detecting and quantitating a

petroleum-degrading bacterium belonging to the genus *Cycloclasticus* using the RNA or DNA probe as claimed in Claim 22.

33. A method for detecting and quantitating a
5 petroleum-degrading bacterium belonging to the genus
Cycloclasticus using the RNA or DNA probe as claimed in Claim
23.

34. A method as claimed in Claim 32, wherein the petroleum-degrading bacterium belonging to the genus *Cycloclasticus* is *Cycloclasticus pugetii* or its closely related species.

35. A method as claimed in Claim 33, wherein the petroleum-degrading bacterium belonging to the genus *Cycloclasticus* is *Cycloclasticus pugetii* or its closely related species.

36. A method for screening a petroleum-degrading bacterium belonging to the genus *Cycloclasticus* using the RNA or DNA probe as claimed in Claim 22.

37. A method for screening a petroleum-degrading
20 bacterium belonging to the genus *Cycloclasticus* using the RNA
or DNA probe as claimed in Claim 23.

38. A method as claimed in Claim 36, wherein the petroleum-degrading bacterium belonging to the genus *Cycloclasticus* is *Cycloclasticus pugetii* or its closely related species.

39. A method as claimed in Claim 37, wherein the petroleum-degrading bacterium belonging to the genus *Cycloclasticus* is *Cycloclasticus pugetii* or its closely related species.

5 40. A method for identifying a petroleum-degrading bacterium belonging to the genus *Cycloclasticus* by means of DNA/DNA or DNA/RNA hybridization using an RNA or DNA probe homologous to SEQ ID NO: 1 or as claimed in Claim 22.

10 41. A method for identifying a petroleum-degrading bacterium belonging to the genus *Cycloclasticus* by means of DNA/DNA or DNA/RNA hybridization using an RNA or DNA probe homologous to SEQ ID NO: 1 or as claimed in Claim 23.

15 42. A method as claimed in Claim 40, wherein the petroleum-degrading bacterium belonging to the genus *Cycloclasticus* is *Cycloclasticus pugetii* or its closely related species.

20 43. A method as claimed in Claim 41, wherein the petroleum-degrading bacterium belonging to the genus *Cycloclasticus* is *Cycloclasticus pugetii* or its closely related species.